The Cytophotometric Estimation of the Desoxyribonucleic Acid (DNA) Content of Individual Plasma Cells from Multiple Myeloma and Nonspecific Plasmacytoses

By Nicholas L. Petrikis and Lois J. Fostad

The histogenesis of plasma cells and their relationship to the plasma cells associated with multiple myeloma has been a subject of considerable controversy. Many authorities consider the plasma cell system to be an independent blood cell series arising from the hematopoietic reticulum cells. Some have designated the plasma cells of multiple myeloma as "myeloma cells" and consider them to be neoplastic forms of plasma cells. Others do not separate myeloma cells from nonspecific plasmacytoses but believe them to be morphologically similar to immature forms of plasmacytes.

Abnormalities of the nucleus, such as multinucleation, giant nuclei, incompletely divided nuclei, and nuclei of unequal size are frequently found in plasma cells, being especially common in multiple myeloma. Of additional interest is observation of the infrequency of mitoses in both forms of plasma cells. The association of such nuclear abnormalities and the scarcity of mitotic figures suggests the presence of abnormal or atypical mitosis in plasma cells. Amitosis has, in the past, been regarded as a common mechanism of cell division in plasma cells.

These considerations and the clinically malignant nature of myeloma cells suggested that a study of the DNA content of plasma cell nuclei might contribute to the available information concerning mitosis in plasma cells and the nature of the myeloma cell. The DNA content of the nucleus has been shown to be intimately related to chromosome structure and mitotic activity, and to be of value as a means of quantitatively detecting alterations in chromosomal and mitotic DNA relationships in certain malignant and leukemic cells. The present report deals with the cytophotometric estimation of the DNA content of individual plasma and myeloma cells at the recognized stages of morphologic differentiation.

Materials and Methods

The plasma cells were studied in smears of aspirated bone marrow from six patients with multiple myeloma, two adults with cirrhosis of the liver, one adult with lupus erythematosus...
tosus, and a child with aplastic anemia. The latter four patients had a large number of
plasmacytes in their bone marrows. The smears were dipped, before drying, into absolute
(100 per cent) methyl alcohol for 30 seconds and then air dried. Following hydrolysis in
1 N HCl for 12 minutes at 56 C., the smears were stained for one hour in the Feulgen reagent.
The cytophotometric apparatus and technics employed were those previously described
by Pollister, and Ornstein,21 Korson,21 and by the authors.17

Light of wave length 550 mM
(20 mM band width) was obtained from the Beckman Model B spectrophotometer and the
exit-slit image was focused onto the stage plane of the microscope. The individual nuclei
were located under oil immersion, their diameters were measured with a filar micrometer,
and their images were projected into the photometric apparatus mounted above the micro-
scope and isolated by means of the iris diaphragm. Measurements of light transmission
through the nuclear plugs and a blank area of equal size were made on each nucleus, and
the percentage transmission calculated. The extinction, or optical density, was calculated
from the formula

\[ E = \log \frac{1}{T} \]

and the amount of DNA-Feulgen material in the nucleus was calculated in arbitrary units by
multiplying the extinction by the nuclear area. A detailed discussion of the cytophotometric technic can be found elsewhere.21

Air-dried marrow smears were made from each patient and were stained with Wright
stain. For additional aid in the identification of myeloma and plasma cells in the marrow
smears where other types of cells were found, the smears were counterstained for five
minutes with Naphthol yellow (0.1 Gm. Naphthol yellow in 1.0 per cent acetic acid). This
dye does not interfere with Feulgen measurements made at 550 mM, the absorption peak
being about 445 mM in the blue portion of the visible spectrum.23 The use of the Beckman
Model B spectrophotometer as the light source made it possible to rapidly change the wave
length of the light between 445 and 550 mM. Plasma cells stained with Naphthol yellow
strongly absorbed light of 445 mM in the areas corresponding to cytoplasmic basophilia
and materially assisted in their identification.

Measurements of DNA in myeloma cell nuclei were occasionally complicated by th

<p>| Table 1.—Average DNA Content (Feulgen Units) in Normal Lymphocytes, Non-Specific Plasmacytoses and Myeloma Cells |
|--------------------------------------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Circulating Lymphocytes</th>
<th>Average DNA (Feulgen Units)</th>
<th>Standard Error</th>
<th>Number of Cells Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4.25</td>
<td>0.13</td>
<td>30</td>
</tr>
<tr>
<td>Normal</td>
<td>3.85</td>
<td>0.08</td>
<td>15</td>
</tr>
<tr>
<td>Non-Specific Plasmacytoses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cirrhosis of liver</td>
<td>4.17</td>
<td>0.22</td>
<td>30</td>
</tr>
<tr>
<td>Cirrhosis of liver</td>
<td>4.05</td>
<td>0.17</td>
<td>24</td>
</tr>
<tr>
<td>Lupus erythematosus</td>
<td>4.48</td>
<td>0.23</td>
<td>30</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>4.35</td>
<td>1.19</td>
<td>35</td>
</tr>
<tr>
<td>Average</td>
<td>4.41</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Multiple Myeloma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case number 1</td>
<td>10.49</td>
<td>1.3</td>
<td>20</td>
</tr>
<tr>
<td>Case number 2</td>
<td>7.62</td>
<td>1.9</td>
<td>32</td>
</tr>
<tr>
<td>Case number 3</td>
<td>9.77</td>
<td>1.2</td>
<td>30</td>
</tr>
<tr>
<td>Case number 4</td>
<td>8.01</td>
<td>1.1</td>
<td>30</td>
</tr>
<tr>
<td>Case number 5</td>
<td>6.75</td>
<td>2.1</td>
<td>30</td>
</tr>
<tr>
<td>Case number 6</td>
<td>8.27</td>
<td>1.3</td>
<td>20</td>
</tr>
<tr>
<td>Average</td>
<td>8.48</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>
presence of markedly enlarged nucleoli in plasmacytes. In such instances, extinction measurements were obtained separately on the portion of the nucleus overlying the nucleolus and the remaining area of the nucleus. The area of this portion of nucleus was calculated from the diameter of the nucleolus. The area of the remaining portion of the nucleus was calculated utilizing the formula for the area of the ring between two circles of \( r_1 \) and \( r^2 \), one of which encloses the other:

\[
\text{Area} = (r_1 + r^2) (r_1 - r^2)
\]

Most of the smears studied were from patients with marked myelomatosis and plasmacytosis and identification of cells of the plasmacytic series was simple. However, classification of Feulgen-stained nuclei of plasma cells as to stage of maturation was frequently difficult since the classification of these stages has been based in part upon staining properties with Romanowsky stains. In the present studies the classification of developmental stages was based on careful comparison studies of Wright stained smears and on such criteria as size of the nucleus; texture of the nuclear chromatin, the presence, size and number of nucleoli, location of the nucleus within the cytoplasm, and the amount of cytoplasm in relation to the nucleus. These criteria were based on the descriptions by Fadem, Diggs and Sirridge, and Moeschlin. Plasmoblasts were characterized by large central nuclei with fine chromatin and having a number of fine nucleoli. In most instances these nuclei were over 12 \( \mu \) in diameter. Plasmacytes had coarser chromatin and 2-3 nucleoli, or frequently a single large nucleolus. The nuclear diameter ranged from 7-12 \( \mu \). The cyto-

![Diagram showing DNA distribution in plasma cells from non-myeloma and multiple myeloma](image-url)

Fig. 1.—The distribution of DNA values in individual plasma cell nuclei from non-specific plasmacytoses (upper graph) and from multiple myeloma (lower graph).
plasmic area was increased over that of the plasmoblast. Plasmacytes had coarse chromatin, minute or absent nucleoli, and an eccentric nucleus. Nuclear diameters were less than 7 μ.

DNA measurements were also made on normal lymphocytes from the peripheral blood of two subjects in order to determine the content of DNA equivalent to a known diploid chromosome number. This value served as a reference standard for the determination of tetraploid (4n) and octoploid (8n) DNA values.

Chromosome counts were not attempted because of the poor definition of the material and the scarcity of mitotic figures in plasma cells.

RESULTS

1. Normal Lymphocytes

The average DNA content per nucleus, in relative units, in circulating lymphocytes is shown in Table 1. These nuclei fell into a single well-defined group with a mean value of 4.05 ± S.E. 0.10. This value was designated as the diploid (2n) DNA value and is shown as a dotted vertical line on the accompanying figures.

![Graph showing DNA values in plasma cell nuclei from non-specific plasma cytoses according to stage of morphologic differentiation.](image-url)
II. Plasma Cells (Non-Myeloma)

The average DNA content per nucleus (relative units) in plasma cells from the marrow of two patients with cirrhosis, one with lupus erythematosus, and one with aplastic anemia are shown in Table 1. The mean DNA value for these plasma cells was $4.41 \pm 0.20$ units and the DNA values fell into a single group. (Figure 1.) The DNA content was not related to nuclear diameter of the plasmacytes. The larger nuclei over 7 m$\mu$ which contained nucleoli were designated as proplasmacytes and had the same average DNA content as the smaller

![Graph showing the distribution of DNA values in myeloma cells according to state of morphologic differentiation.](https://www.bloodjournal.org/content/2/8/1208/F1.large.jpg)
Table 2.—DNA Content of the Individual Nuclei in Binucleated Myeloma Cells

<table>
<thead>
<tr>
<th>Cell</th>
<th>Nucleus No. 1</th>
<th>Nucleus No. 2</th>
<th>Total DNA cell</th>
<th>Cell</th>
<th>Nucleus No. 1</th>
<th>Nucleus No. 2</th>
<th>Total DNA cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.3</td>
<td>8.1</td>
<td>15.4</td>
<td>10</td>
<td>3.57</td>
<td>8.92</td>
<td>12.49</td>
</tr>
<tr>
<td>2</td>
<td>10.0</td>
<td>5.4</td>
<td>15.4</td>
<td>11</td>
<td>7.24</td>
<td>6.74</td>
<td>13.98</td>
</tr>
<tr>
<td>3</td>
<td>11.0</td>
<td>9.0</td>
<td>20.0</td>
<td>12</td>
<td>4.78</td>
<td>5.43</td>
<td>11.21</td>
</tr>
<tr>
<td>4</td>
<td>8.9</td>
<td>8.2</td>
<td>17.1</td>
<td>13</td>
<td>4.47</td>
<td>3.84</td>
<td>8.31</td>
</tr>
<tr>
<td>5</td>
<td>23.3</td>
<td>4.5</td>
<td>27.8</td>
<td>14</td>
<td>5.3</td>
<td>3.3</td>
<td>8.6</td>
</tr>
<tr>
<td>6</td>
<td>10.7</td>
<td>18.8</td>
<td>29.5</td>
<td>15</td>
<td>6.45</td>
<td>5.51</td>
<td>11.96</td>
</tr>
<tr>
<td>7</td>
<td>4.4</td>
<td>4.1</td>
<td>8.51</td>
<td>16</td>
<td>8.41</td>
<td>12.40</td>
<td>20.81</td>
</tr>
<tr>
<td>8</td>
<td>8.7</td>
<td>6.8</td>
<td>15.56</td>
<td>17</td>
<td>9.54</td>
<td>8.77</td>
<td>18.31</td>
</tr>
<tr>
<td>9</td>
<td>6.36</td>
<td>3.72</td>
<td>10.08</td>
<td>18</td>
<td>7.18</td>
<td>6.90</td>
<td>14.08</td>
</tr>
</tbody>
</table>

(less than 7 μm) plasmacytes. The distribution curves for these morphologic stages were essentially similar. (Figure 2.)

III. Myeloma Plasma Cells

The average DNA content per nucleus, in relative units, of plasma cells from myeloma patients is shown in Table 1. While the mean value for the DNA content of these cells was 8.48 ± 1.5 units, the distribution curve revealed a wide spread of the individual nuclear values, ranging from those equal to normal lymphocytes to four times this amount. (Figure 1.) When the DNA contents of myeloma nuclei were grouped according to morphologic stage of development, three distinct classes of DNA were found corresponding to plasmacytes, proplasmacytes and plasmoblasts. (Figure 3.) The myeloma plasmacytes contained DNA values similar to normal lymphocytes and plasmacytes of non-myeloma sources. Myeloma proplasmacytes contained approximately four times the DNA content of mature plasmacytes. Overlapping of the three morphologic groups was observed.

The DNA content was determined in the individual nuclei in 18 binucleated myeloma plasma cells. The total DNA content of the myeloma cell and the amount contained in the individual nuclei of each cell are shown in Table 2. Considerable variation in DNA content was found among the nuclei in multinucleated cells. Approximately equal amounts of DNA were present in 11 cells and unequal distribution was found in 7 cells. However, only 3 cells contained nuclei with diploid DNA values. Most of the nuclei had a DNA content twice that of the lymphocyte and plasmacyte content. In a few instances grossly unequal DNA distributions occurred and were morphologically evident as nuclei of unequal size. The sum of the DNA content of both nuclei in the binucleated cells fell nearer the highest levels of DNA observed in plasmoblasts.

DISCUSSION

The results of the present study indicate that the content of DNA in individual plasmacytes and proplasmacytes from non-specific plasmacytoses is similar in amount to that present in normal circulating lymphocytes. It may therefore be concluded that the plasmacytes are diploid (2n) with respect to chromosome number, since lymphocytes are shown to contain the diploid chromosome number and have been demonstrated to contain twice the DNA content of haploid (1n)
spermatids. Plasmoblasts were not encountered among the non-specific plasmacytes studied; they are said to be seldom if ever observed in non-myelomatous patients.

Of considerable interest was the finding of an increased content of DNA in the majority of myeloma plasma cell nuclei, which appeared to be related to the morphologic stage of differentiation. Myeloma plasmacytes contained a DNA content equal to the diploid quantity of DNA, myeloma proplasmacytes contained approximately twice the diploid quantity of DNA (tetraploid), and myeloma plasmoblasts contained four times the diploid quantity of DNA (octoploid). Overlapping of the DNA values of the three morphologic stages was found and was attributed to the difficulty in classifying the cells as to exact stage of differentiation.

The present data appear to demonstrate a geometric DNA series corresponding to the three morphologic stages of the myeloma plasma cell classification. It is not possible to state whether such increased quantities of DNA are a result of mitotic activity, reduplication of the chromosome numbers (polyploidy), or internal reduplication of the DNA chromonema strands of the chromosomes (polyteny).

The tetraploid and near-tetraploid values in myeloma proplasmacytes may represent a premitotic build up of DNA, which might be anticipated in view of the malignant clinical nature of multiple myeloma. The scarcity of mitotic figures does not lend support to this possibility, but data on the intermitotic interval of myeloma cells are needed to establish this point, since the cytophotometric technic of DNA measurement does not give information on the rate of DNA accumulation. It has been reported that plasma cells are diploid, but the data included do not give information as to chromosome numbers. Undritz describes plasma cell chromosomes as being thick, and photographs demonstrate little resemblance to the well-defined mitotic figures seen in the erythrocytic series.

In the event that the plasma cells of multiple myeloma contain the diploid chromosome number, it can be inferred from the present data that myeloma plasmacytes and plasmoblasts contain polytene chromosomes. Such chromosomes have been described in animal tumors and in human mammary carcinoma.

Fankhauser has discussed the possible mechanisms of formation of polyploid and polytene nuclei. These consist of (1) failure of cytoplasmic division following nuclear division, with fusion of the chromosomes of the two nuclei at the next mitosis, (2) complete or partial failure of formation of the spindle apparatus in the metaphase, resulting in incomplete or partial assortment of the chromosomes, (3) endomitotic reduplication of chromosome numbers or chromonemata without dissolution of the nuclear membrane and the formation of the mitotic spindle apparatus. Bloch has experimentally demonstrated the formation of nuclear DNA classes in fibroblast cultures with colchicine and Levan and Hausecha have described endomitotic mechanisms in ascites tumors. The presence of such abnormalities of mitosis would be reflected in the nuclear DNA content as deviations from the diploid DNA quantity. The occurrence of a polyploid DNA series in myeloma cells and the markedly abnormal content of DNA in the bimucleated plasma cells studies can be interpreted as resulting from the presence of such mechanisms.
DNA analyses were not made on the reticulum precursor cells due to the difficulty in identifying these cells with the Feulgen stain. The reticulum precursor cell to the normal plasmacyte would presumably be a diploid somatic cell. In myeloma the reticulum cells are stated to be pathologic.33 During the process of differentiation the reticulum cell would need to acquire an amount of DNA equivalent to the octoploid value in the plasmoblast and to be followed by a reduction of DNA content during the subsequent process of differentiation toward the plasmacyte stage. The absence of reduction divisions are evidence against this hypothesis, although a progressive decrease in DNA content has been reported to occur in the maturation of the erythroid cells.22 An alternative and more likely possibility is that myeloma cells are malignant or aberrant forms of plasma cells as claimed previously47 and as reflected by their increased DNA content, and further, that the usual morphologic scheme of differentiation ascribed to plasma cells does not pertain to myeloma cells. The latter interpretation supports the designation of the plasma cells of multiple myeloma as "myeloma cells." Irrespective of the mechanism of formation of the increased DNA content, the present findings indicate a basic alteration in the quantitative DNA-chromosome relationship in plasma cells of multiple myeloma and offer cytochemical support for the separation of these cells from the plasma cells found in non-specific plasmacytoses.

**Summary**

The cytophotometric technic employing the Feulgen reagent was used to determine the relative amount of DNA in the nuclei of individual myeloma and plasma cells aspirated from the bone marrow. Individual myeloma cells were found to contain markedly elevated amounts of DNA as compared to that in the plasma cells of non-specific plasmacytoses. Myeloma cells were found to contain geometric multiples (2, 4, 8) of the amount of DNA present in normal lymphocytes, and the degree of DNA "ploidy" was related to the morphologic stage of immaturity. Polyploid DNA values were not found in the non-specific plasmacytoses. The possible mechanisms of formation were discussed. The findings are interpreted as suggesting the presence of a basic alteration in the quantitative DNA-chromosome relationship in myeloma cells, and they offer additional support for the separation of the myeloma plasmacytoses from non-specific plasmacytoses.

**Summario in Interlingua**

Le technica cytophotometric, utilizante le reagentem de Feulgen, esseva empleate pro determinar le relative quantitate de acido disoxyribonucleic in le nucleos de cellulas plasmatic e myelomatic individual, obtenite per aspiration ab le medulla ossee. Esseva constatate que cellulas myelomatic individual contineva marcatemente elevate quantitates de acido disoxyribonucleic in comparation con le quantitates trovate in le cellulas plasmatic de plasmacytoses non-specific. Le cellulas myelomatic contineva multiples geometric del quantitate de acido disoxyribonucleic presente in normal lymphocytes, e le grado del polyplodia de acido disoxyribonucleic esseva relacionate al stadio morphologic de immaturitate. Valores polyploide de acido disoxyribonucleic non esseva trovate in le caso del plasmacytoses non-specific. Le mechanismos possibile del formation es discutite.
Secundo nuestra interpretación, los datos obtenidos en este estudio indican la presencia de un alteración fundamental en la relación cuantitativa inter acido desoxiribonucleic e y cromosomas en células mieloma. Los datos representan un soporte adicional para la concepción que las plasmaicitas mieloma debe ser separada de las plasmaicitas no-específicas.

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